Increased Concentrations of Prostaglandin D_2 During Post-Fracture Bone Remodeling

MAXIME A. GALLANT, ESTELLE CHAMOUX, MARTINE BISSON, CATARINA WOLSEN, JEAN-LUC PARENT, SOPHIE ROUX, and ARTUR J. de BRUM-FERNANDES

ABSTRACT. ***Objective.*** To test the hypothesis that increased concentrations of prostaglandin D_2 (PGD_2) correlate with bone remodeling. Studies using isolated bone cells indicate that PGD_2 may be implicated in the regulation of bone homeostasis, with a positive influence on bone anabolism. We studied patients with traumatic fractures and age- and sex-matched healthy controls as an *in vivo* model of increased bone remodeling.

Methods. Thirty-five patients with bone fracture and matched controls were recruited. Urine and sera samples were collected. Urinary 11ß-$PGF_{2\alpha}$, a PGD_2 metabolite, and PGE_2 metabolites (PGEM), serum lipocalin-type PGD_2 synthase (L-PGDS), bone alkaline phosphatase (bone ALP), and crosslinked C-telopeptides of type I collagen (CTX) were measured.

Results. At 5–6 weeks post-fracture, 11ß-$PGF_{2\alpha}$, L-PGDS, bone ALP, and CTX were significantly increased in the fracture patients compared to controls. PGEM levels were not different between groups. Levels of 11ß-$PGF_{2\alpha}$ and bone ALP were positively correlated, suggesting that PGD_2 may be implicated in fracture repair.

Conclusion. These results support our working hypothesis that PGD_2 could be implicated in the control of bone anabolism in humans. (First Release Jan 15 2010; J Rheumatol 2010;37:644–9; doi:10.3899/jrheum.090622)

Key Indexing Terms:
BONE PROSTAGLANDIN D_2 PROSTAGLANDIN SYNTHASE
FRACTURE REPAIR BONE REMODELING

Fracture repair is a physiological process where inflammation, angiogenesis, and bone remodeling play important roles. A number of local and systemic factors influence the fracture repair process: these include cytokines, growth factors, and lipid mediators, which tightly regulate the action of cells responsible for bone healing[1-3].

Prostaglandins (PG) strongly influence the process of fracture repair, as demonstrated by delayed repair in mice null for cyclooxygenase-2 (COX-2)[4] and by animal studies where COX inhibitors were used[5,6]. However, despite the common use of nonsteroidal antiinflammatory drugs (NSAID) post-surgery and post-fracture, the effects of COX inhibition, if any, remain unclear during fracture repair in humans[7]. Some studies suggest that COX inhibition may delay fracture healing or increase the rate of non-unions[8,9], but prospective and controlled clinical trials are still lacking. PG may thus play a role in human bone metabolism, remodeling, and repair.

PG act on osteoblasts and osteoclasts, the cells responsible for bone formation and resorption, respectively[10]. PGD_2 is mostly known for its effects on the immune system and sleep[11,12], but recent evidence suggests new roles for this PG in bone physiology. We demonstrated that human osteoblasts produce PGD_2, and express the functional PGD_2 receptors D protanoid receptor type 1 (DP) and chemoattractant receptor-homologous molecule expressed on T helper cell type 2 (CRTH2). Activation of both receptors can lead to a different osteoblast phenotype, as DP receptor activation decreases osteoprotegerin (OPG) secretion, while CRTH2 increases osteoblast migration and decreases expression of RANKL (receptor activator of nuclear factor-κB ligand)[13]. Moreover, PGD_2 and its metabolites are potent inducers of collagen synthesis by human osteoblasts in culture[14,15]. Recently, we showed that PGD_2 inhibits both human osteoclast differentiation and mature osteoclast activity, reducing bone resorption *in vitro*[16]. These results suggest that PGD_2 may have a physiological role in bone remodeling, favoring bone anabolism.

PGD_2 is produced by 2 terminal PGD_2 synthases: hematopoietic-type synthase (H-PGDS) or lipocalin-type (L-PGDS) synthase. H-PGDS is strongly expressed by immune cells, while L-PGDS is mostly expressed in the

From the Division of Rheumatology, Faculté de médecine et des sciences de la santé, Centre de Recherche Clinique Etienne-Le Bel, Université de Sherbrooke, Sherbrooke, Quebec, Canada.

Supported by grants from the Canadian Institutes of Health Research. Dr. Gallant received a doctoral fellowship from the FRSQ. Dr. Parent and Dr. Roux receive scholarships from the FRSQ.

M.A. Gallant, PhD(c); E. Chamoux, PhD; M. Bisson, RT; C. Wolsen, BSc; J-L. Parent, PhD; S. Roux, MD, PhD; A.J. de Brum-Fernandes, MD, PhD, Division of Rheumatology, Université de Sherbrooke.

Address correspondence to Dr. A.J. de Brum-Fernandes, Division of Rheumatology, Université de Sherbrooke, 3001, 12th Avenue North, Sherbrooke, QC J1H 5N4. E-mail: Artur.Fernandes@USherbrooke.ca

Accepted for publication October 4, 2009.

brain and heart[17]. In addition to PGD_2 synthesis, L-PGDS also acts as a transporter for small lipophilic ligands such as retinoids and thyroid hormones. Altered expression of L-PGDS has been related to detached retinas[18], atherosclerosis[19], sleep disorders[20-22], and impaired renal function in diabetes[23,24]. However, no evidence in the literature links this enzyme to human bone metabolism.

Our study is based on the hypothesis that PGD_2 is an important anabolic autacoid implicated in bone repair, as suggested by the *in vitro* studies using isolated osteoblasts and osteoclasts. Should this hypothesis be true, we would expect PGD_2 production to be increased *in vivo* during situations of increased bone remodeling. We studied patients with traumatic fractures and compared them to healthy age- and sex-matched controls as a model of increased bone turnover to test this hypothesis. Specifically, we determined the levels of a stable PGD_2 metabolite and of L-PGDS in patients within 5–6 weeks post-fracture, a period of active bone remodeling, and compared these levels to healthy controls.

MATERIALS AND METHODS

Patients. Patients with traumatic bone fracture were recruited 5–6 weeks post-fracture at the Centre Hospitalier Universitaire de Sherbrooke (CHUS) in collaboration with the Division of Orthopaedic Surgery during their routine visit at the hospital. Patients gave informed consent and the study was approved by the institution's Ethics Review Board. Age-, sex-, and weight-matched healthy controls were recruited at the same center and Bishop's University (Sherbrooke, QC, Canada). Patients and controls were excluded from the study if they used NSAID or corticosteroids less than 7 days before enrolment, if they had ever received calcitonin, bisphosphonates or chemotherapy treatments, or if they suffered from kidney failure, diabetes or asthma.

Blood and urine samples. Urine and blood samples were collected at the time of recruitment. Urine samples were immediately centrifuged at 1500 ×g for 15 min and the supernatants were frozen at –80°C until analyzed. Whole blood was drawn by standard venipuncture without anticoagulants, allowed to coagulate for 30 min at room temperature, and the serum was separated by centrifugation at 1500 ×g for 15 min. Serum aliquots were frozen at –80°C until analyzed.

Biochemical markers. PGD_2 production was assessed by measuring the levels of the PGD_2 primary stable metabolite 11ß-$PGF_{2\alpha}$ in urine samples using enzyme immunoassay (EIA) kits (Cayman Chemical Co., Ann Arbor, MI, USA) with a detection limit of 5.5 pg/ml. This assay specifically recognizes 11ß-$PGF_{2\alpha}$ with less than 0.01% cross-reaction towards PGD_2 or PGE_2.

PGE_2 production was assayed by measuring a stable PGE_2 metabolite (PGEM) after chemical derivatization of PGE_2 and its primary metabolites, namely 13,14-dihydro-15-keto PGE_2 and 13,14-dihydro-15-keto PGA_2, in the standards and urine samples to the single PGEM compound. PGEM concentrations were determined using commercial EIA kits (Cayman Chemical) following the manufacturer's protocol, with a detection limit of 2 pg/ml.

Lipocalin-type PGD_2 synthase expression was determined in the serum using the Prostaglandin D Synthase (lipocalin-type; human) EIA dosage kit (Cayman Chemical; detection limit 2 ng/ml), according to the manufacturer's protocol.

Bone formation occurring during fracture repair was assessed by serum bone-specific alkaline phosphatase (bone ALP) measured by commercial ELISA (Metra® BAP, from Quidel, distributed by Medicorp Inc., Montreal, QC, Canada) on thawed serum aliquots. This ELISA detects as low as 0.7 U/l bone ALP with 3%–8% cross-reactivity with liver ALP and 0.4% towards intestine ALP.

To assess bone resorption, serum C-terminal telopeptides of type 1 collagen (CTX) were measured using the Serum CrossLaps® ELISA (Nordic Bioscience Diagnostics; Medicorp Inc., Montreal, QC, Canada). This ELISA has a detection limit of 0.020 ng/ml of CTX in a linear range up to 3.380 ng/ml.

Urine creatinine was measured (Vitros 950 System Chemistry, Ortho Clinical Diagnostics, Johnson & Johnson) and used to correct urine PG values.

Statistical analyses. Statistical analyses were performed using GraphPad Prism version 5.01. The Wilcoxon signed-rank test was used to compare patients to paired controls, while nonparametric Spearman correlations were used between markers. Data are shown as means ± standard error. Results were considered significant when $p < 0.05$.

RESULTS

Our study was designed to assess possible changes in the stable PGD_2 metabolite 11ß-$PGF_{2\alpha}$ and L-PGDS in patients during the active bone formation phase of fracture repair. A cohort of 35 patients and matched controls was enrolled at our local hospital. No demographic differences were observed between the patients and controls (Table 1).

Prostaglandin D_2 pathway is specifically increased in fracture repair. To assess PGD_2 production, we measured the concentration of 11ß-$PGF_{2\alpha}$ in urine samples. As shown in Figure 1, patients with fractures showed higher levels of 11ß-$PGF_{2\alpha}$ in urine compared to controls (77.4 ± 8.8 vs 49.5 ± 3.9 ng/mmol creatinine, respectively; $p = 0.0023$). Figure 2 shows that serum L-PGDS in patients with fracture was significantly higher than that in controls (0.613 ± 0.03 vs 0.536 ± 0.03 μg/ml, respectively; $p = 0.027$). Since increases in PGD_2 and L-PGDS could have resulted from nonspecific inflammation associated with the fracture we also determined the concentration of stable PGE_2 metabolite, PGEM, in our population. As shown in Figure 3, PGEM did

Table 1. Demographic data of the study cohort.

Feature	Controls	Patients
No.	35	35
Sex, M/F	17/18	17/18
Age, yrs, mean (range)	34.8 ± 11.9 (20–56)	35 ± 11.8 (18–54)
Weight, kg, mean (range)	68.4 ± 11.6 (49.9–97.5)	68.3 ± 14.1 (44–118)
Fracture type		
Long bones		N = 25 (71%) Tibia/fibula 10 Radius/ulna 7 Humerus 4 Femur 2 Polytrauma 2
Other		N = 10 (29%) Hand/foot 4 Vertebrae 3 Hip 2 Scapula 1

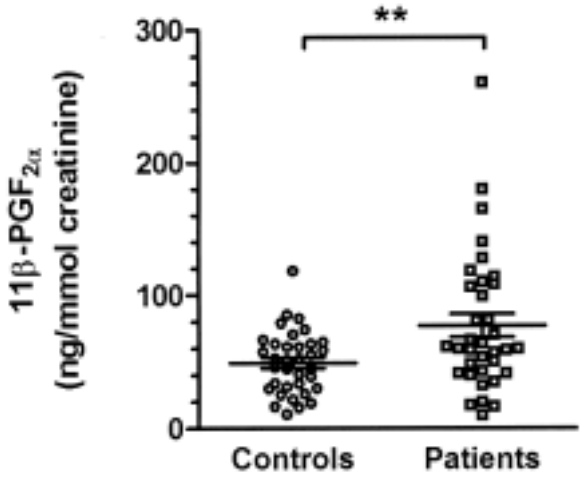

Figure 1. Urine 11ß-$PGF_{2\alpha}$ was measured in patients and controls and corrected for creatinine. Data are means ± SEM. **p < 0.01.

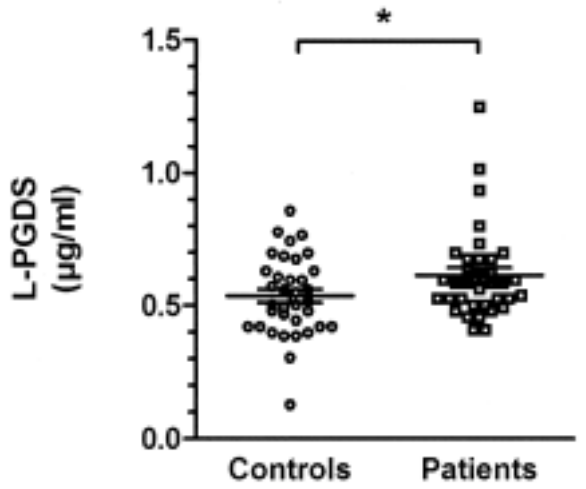

Figure 2. Serum lipocalin-type PGD_2 (L-PGDS) synthase was measured in patients and controls. Data are means ± SEM. *p < 0.05.

not differ significantly between control and patient groups (98.7 ± 12.5 vs 118.0 ± 18.8 ng/mmol creatinine, respectively; p = 0.3633), therefore arguing against a nonspecific overall increase in PG production.

Bone markers. Fracture repair is a process where both bone formation and resorption occur. Serum bone ALP and CTX were measured to assess these 2 phenomena, respectively. As expected, level of serum bone ALP was significantly higher in patients compared to controls (31.3 ± 2.6 U/l vs 24.4 ± 1.7 U/l, respectively; p = 0.0033; Figure 4). A strong increase in the bone resorption marker CTX was also observed in patients compared to controls (0.568 ± 0.07 ng/ml vs 0.301 ± 0.05 ng/ml, respectively; p = 0.0002; Figure 5), thus confirming active bone remodeling in the fracture group.

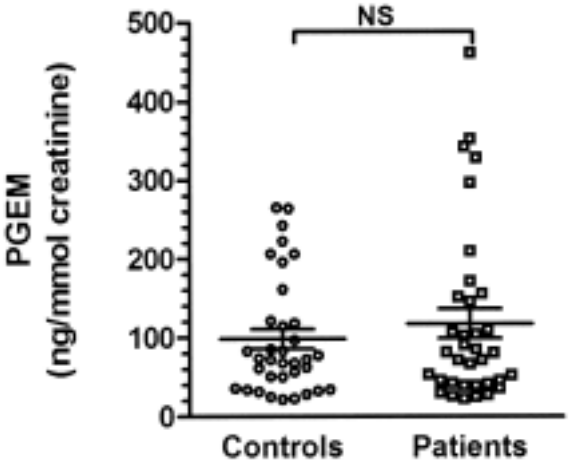

Figure 3. Urine PGE_2 metabolite (PGEM) was measured in patients and controls and corrected for urine creatinine. Data are means ± SEM. NS: not significant.

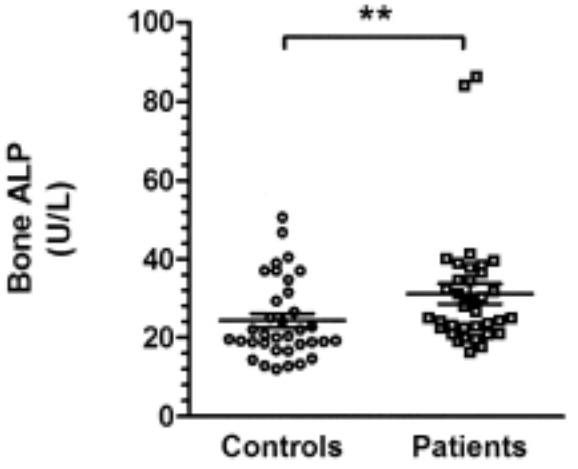

Figure 4. Serum bone ALP was measured in patients and controls. Data are means ± SEM. **p < 0.01.

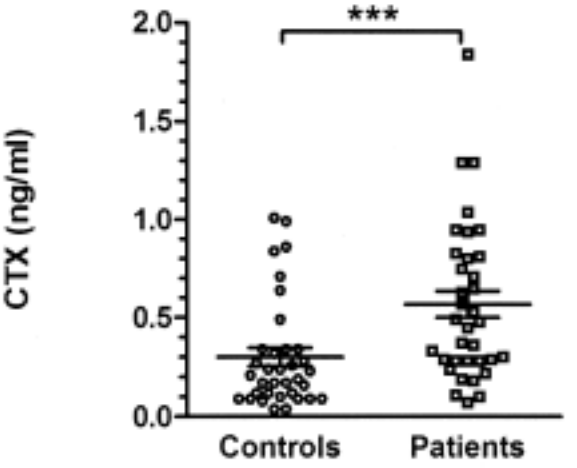

Figure 5. Serum CTX was measured in patients and controls. Data are means ± SEM. ***p < 0.001.

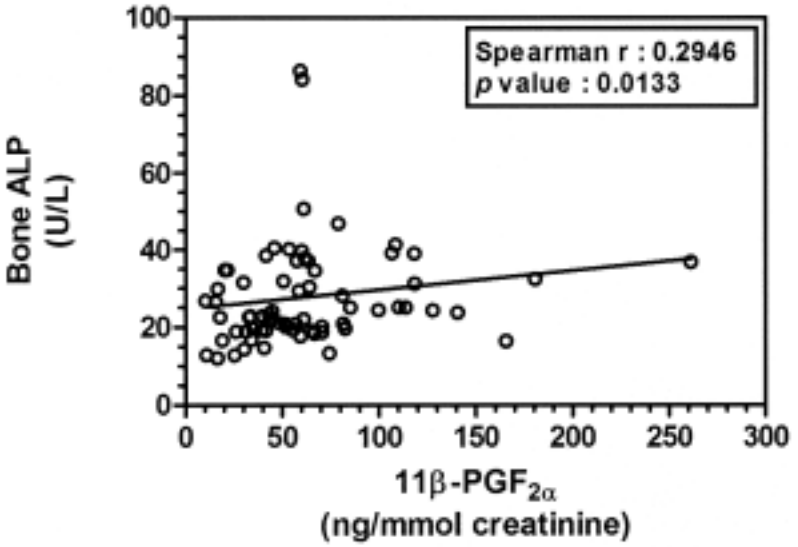

Figure 6. Spearman's rank test was performed to test correlation between 11ß-$PGF_{2\alpha}$ and bone ALP in the whole cohort (N = 70).

Correlation of PGD_2 metabolite and bone formation marker. To determine if the higher production of the PGD_2 metabolite 11ß-$PGF_{2\alpha}$ in the patient group would correlate to an increase in the bone remodeling process, we tested whether serum bone ALP correlated with urine 11ß-$PGF_{2\alpha}$. As shown in Figure 6, bone ALP and 11ß-$PGF_{2\alpha}$ levels were positively correlated (Spearman r = 0.2946, p = 0.0133). In contrast, no correlation was observed between 11ß-$PGF_{2\alpha}$ and CTX. The expression of L-PGDS and level of PGEM were not correlated with any marker measured.

DISCUSSION

Bone metabolism is a complex and tightly controlled process implicating several cytokines, growth factors, and soluble mediators such as prostaglandins[1,3]. Inadequate control of the balance between bone production and resorption, either locally or systemically, is an important pathophysiologic sequence in different diseases such as rheumatoid arthritis[25], spondyloarthritis[26], osteoarthritis[27], periodontitis[28], and osteoporosis[29], to cite a few. Better understanding of the different elements implicated in this control may lead to identification of new pharmacological targets for treatment of these diseases.

Evidence in the literature from our group[13,16] and others[14,15] suggests that PGD_2 may have a positive influence on bone metabolism, increasing bone formation and inhibiting bone resorption. Our objective was to test *in vivo* the hypothesis that increased levels of PGD_2 would be associated with increased bone remodeling using traumatic bone fracture in humans as an experimental model.

Fracture repair is a physiological event regulated by local and systemic factors. Immediately after a fracture, the microenvironment of the damaged site is drastically changed. Formation of hematoma and inflammation are the first events to occur. During this period, leukocytes are attracted to the wound site, where they secrete various mediators modulating the inflammatory response, including cytokines and PG. While this inflammatory response lasts only a few days, PG-mediated effects may last longer as they remain actively produced by bone and surrounding cells[13,30,31].

The importance of PG in the process of fracture repair is well characterized in animal models. COX-2 was shown to be essential to fracture repair[4,6], while PGE_2 receptors EP2 and EP4 were shown to be implicated in murine fracture repair[32,33], but the role of PGD_2 in fracture repair and bone remodeling remains unknown.

We investigated if L-PGDS expression and the stable urinary PGD_2 metabolite 11ß-$PGF_{2\alpha}$ were changed during the process of bone remodeling and if they correlated with markers of bone formation and resorption. 11ß-$PGF_{2\alpha}$ is the major PGD_2 metabolite produced *in vivo* and is considered a physiological marker of PGD_2 production: it has been used to assess mast cell activation and PGD_2 production during allergic and asthmatic responses[34-36]. We demonstrated that 11ß-$PGF_{2\alpha}$ is significantly increased during fracture repair. To our knowledge, this is the first time that PGD_2 has been linked to bone metabolism in humans.

To test whether this key enzyme was implicated in bone remodeling, serum L-PGDS was measured in patients with fractures. Like 11ß-$PGF_{2\alpha}$, L-PGDS was significantly increased during fracture repair. It is interesting that slight changes in serum L-PGDS can be a predictor or marker of pathologies such as coronary heart disease[19], hypertension[37], sleep apnea[20], and diabetes[23,24]. We cannot rule out

the possible contribution of the other PGD_2 synthase, H-PGDS, in the process, but technical limitations prevent measurement of this intracellular enzyme in blood or other body fluids.

PGD_2 is a key component of the inflammatory response[38,39]. Therefore, all patients were studied during an orthopedic followup visit 5 to 6 weeks post-fracture, a time at which inflammation is not considered to be a major component of the repair process[40] and when bone remodeling starts to occur[41]. We found no significant difference in the stable PGE_2 metabolite in patients compared to controls. This suggests that the increased 11ß-$PGF_{2\alpha}$ levels observed in patients were not due to a general increase in PG synthesis associated with inflammation. Since osteoblasts were shown to produce PGD_2, it is tempting to hypothesize that osteoblasts are responsible for the increased synthesis of PGD_2 found in patients with fracture; this study, however, was not designed to allow identification of the cellular origin of PGD_2.

Bone remodeling is often measured by biomarkers of bone turnover. During fracture repair, both endochondral and intramembranous bone formation occur, leading to formation of a bony and cartilaginous callus, which will later be resorbed in order for the bone to regain its previous integrity, resistance, and shape. Activation of osteoblasts and osteoclasts is critical for the completion of fracture repair. We measured serum levels of bone ALP, as a marker of bone formation and osteoblast activation, as well as serum CTX, to assess bone resorption. Concentrations of both markers were increased in the fracture patients compared to controls, indicating that there was significantly increased bone turnover in these patients. This confirms the premise that increased bone remodeling is occurring in the studied population. To test our hypothesis that PGD_2 is increased during bone remodeling, we tested if 11ß-$PGF_{2\alpha}$ correlated with levels of either bone ALP or CTX. Our analysis showed a significant and positive correlation with bone ALP and no correlation with CTX. Moreover, no correlation was found for the bone markers and PGEM, thus underlying the specificity of PGD_2 production during fracture repair.

Our results show the first evidence that a specific prostaglandin pathway, PGD_2 and its lipocalin-type synthase, is activated during fracture repair in humans. Moreover, we demonstrated that PGD_2 production correlates with a classic osteoblast activation and bone formation marker. These findings support our hypothesis that PGD_2 may be an important player in bone physiology and that it may constitute an important and biologically relevant anabolic stimulus for bone.

ACKNOWLEDGMENT

The authors thank the Division of Orthopaedic Surgery, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, for help in patient recruitment.

REFERENCES

1. Szczesny G. Molecular aspects of bone healing and remodeling. Pol J Pathol 2002;53:145-53.
2. Bahar H, Benayahu D, Yaffe A, Binderman I. Molecular signaling in bone regeneration. Crit Rev Eukaryot Gene Expr 2007;17:87-101.
3. Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. Injury 2005;36:1392-404.
4. Zhang X, Schwarz EM, Young DA, Puzas JE, Rosier RN, O'Keefe RJ. Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. J Clin Invest 2002;109:1405-15.
5. Gerstenfeld LC, Thiede M, Seibert K, Mielke C, Phippard D, Svagr B et al. Differential inhibition of fracture healing by non-selective and cyclooxygenase-2 selective non-steroidal anti-inflammatory drugs. J Orthop Res 2003;21:670-5.
6. Simon AM, Manigrasso MB, O'Connor JP. Cyclo-oxygenase 2 function is essential for bone fracture healing. J Bone Miner Res 2002;17:963-76.
7. Vuolteenaho K, Moilanen T, Moilanen E. Non-steroidal anti-inflammatory drugs, cyclooxygenase-2 and the bone healing process. Basic Clin Pharmacol Toxicol 2008;102:10-4.
8. Bhattacharyya T, Levin R, Vrahas MS, Solomon DH. Nonsteroidal antiinflammatory drugs and nonunion of humeral shaft fractures. Arthritis Rheum 2005;53:364-7.
9. Giannoudis PV, MacDonald DA, Matthews SJ, Smith RM, Furlong AJ, De Boer P. Nonunion of the femoral diaphysis. The influence of reaming and non-steroidal anti-inflammatory drugs. J Bone Joint Surg Br 2000;82:655-8.
10. Raisz LG. Prostaglandins and bone: physiology and pathophysiology. Osteoarthritis Cartilage 1999;7:419-21.
11. Kostenis E, Ulven T. Emerging roles of DP and CRTH2 in allergic inflammation. Trends Mol Med 2006;12:148-58.
12. Hayaishi O. Molecular genetic studies on sleep-wake regulation, with special emphasis on the prostaglandin D(2) system. J Appl Physiol 2002;92:863-8.
13. Gallant MA, Samadfam R, Hackett JA, Antoniou J, Parent JL, de Brum-Fernandes AJ. Production of prostaglandin D(2) by human osteoblasts and modulation of osteoprotegerin, RANKL, and cellular migration by DP and CRTH2 receptors. J Bone Miner Res 2005;20:672-81.
14. Koshihara Y, Amano T, Takamori R. Prostaglandin D2 stimulates calcification by human osteoblastic cells. Adv Prostaglandin Thromboxane Leukot Res 1991;21B:847-50.
15. Tasaki Y, Takamori R, Koshihara Y. Prostaglandin D2 metabolite stimulates collagen synthesis by human osteoblasts during calcification. Prostaglandins 1991;41:303-13.
16. Durand M, Gallant MA, de Brum-Fernandes AJ. Prostaglandin D2 receptors control osteoclastogenesis and the activity of human osteoclasts. J Bone Miner Res 2008;23:1097-105.
17. Urade Y, Eguchi N. Lipocalin-type and hematopoietic prostaglandin D synthases as a novel example of functional convergence. Prostaglandins Other Lipid Mediat 2002;68-69:375-82.
18. Jaggi GP, Flammer J, Huber AR, Killer HE. Lipocalin-like prostaglandin D synthase in subretinal fluid of detached retinas in humans. Retina 2008;28:858-63.
19. Inoue T, Eguchi Y, Matsumoto T, Kijima Y, Kato Y, Ozaki Y, et al. Lipocalin-type prostaglandin D synthase is a powerful biomarker for severity of stable coronary artery disease. Atherosclerosis 2008;201:385-91.
20. Barcelo A, de la Pena M, Barbe F, Pierola J, Bosch M, Agusti AG. Prostaglandin D synthase (beta trace) levels in sleep apnea patients with and without sleepiness. Sleep Med 2007;8:509-11.
21. Qu WM, Huang ZL, Xu XH, Aritake K, Eguchi N, Nambu F, et al. Lipocalin-type prostaglandin D synthase produces prostaglandin D2

involved in regulation of physiological sleep. Proc Natl Acad Sci USA 2006;103:17949-54.
22. Jordan W, Tumani H, Cohrs S, Eggert S, Rodenbeck A, Brunner E, et al. Prostaglandin D synthase (beta-trace) in healthy human sleep. Sleep 2004;27:867-74.
23. Yoshikawa R, Wada J, Seiki K, Matsuoka T, Miyamoto S, Takahashi K, et al. Urinary PGDS levels are associated with vascular injury in type 2 diabetes patients. Diabetes Res Clin Pract 2007;76:358-67.
24. Hirawa N, Uehara Y, Ikeda T, Gomi T, Hamano K, Totsuka Y, et al. Urinary prostaglandin D synthase (beta-trace) excretion increases in the early stage of diabetes mellitus. Nephron 2001;87:321-7.
25. Schett G. Review: Immune cells and mediators of inflammatory arthritis. Autoimmunity 2008;41:224-9.
26. Huang W, Schwarz EM. Mechanisms of bone resorption and new bone formation in spondyloarthropathies. Curr Rheumatol Rep 2002;4:513-7.
27. Goldring SR. The role of bone in osteoarthritis pathogenesis. Rheum Dis Clin North Am 2008;34:561-71.
28. Cochran DL. Inflammation and bone loss in periodontal disease. J Periodontol 2008;79:1569-76.
29. Mundy GR. Osteoporosis and inflammation. Nutr Rev 2007;65:S147-51.
30. Hackett JA, Allard-Chamard H, Sarrazin P, de Fatima Lucena M, Gallant MA, Fortier I, et al. Prostaglandin production by human osteoclasts in culture. J Rheumatol 2006;33:1320-8.
31. Dekel S, Lenthall G, Francis MJ. Release of prostaglandins from bone and muscle after tibial fracture. An experimental study in rabbits. J Bone Joint Surg Br 1981;63-B:185-9.
32. Paralkar VM, Borovecki F, Ke HZ, Cameron KO, Lefker B, Grasser WA, et al. An EP2 receptor-selective prostaglandin E2 agonist induces bone healing. Proc Natl Acad Sci USA 2003;100:6736-40.
33. Li M, Healy DR, Li Y, Simmons HA, Crawford DT, Ke HZ, et al. Osteopenia and impaired fracture healing in aged EP4 receptor knockout mice. Bone 2005;37:46-54.
34. Bochenek G, Nizankowska E, Gielicz A, Swierczynska M, Szczeklik A. Plasma 9α,11ß-PGF2, a PGD2 metabolite, as a sensitive marker of mast cell activation by allergen in bronchial asthma. Thorax 2004;59:459-64.
35. Bochenek G, Nagraba K, Nizankowska E, Szczeklik A. A controlled study of 9α,11ß-PGF2 (a prostaglandin D2 metabolite) in plasma and urine of patients with bronchial asthma and healthy controls after aspirin challenge. J Allergy Clin Immunol 2003;111:743-9.
36. Nagakura T, Obata T, Shichijo K, Matsuda S, Sigimoto H, Yamashita K, et al. GC/MS analysis of urinary excretion of 9α, 11ß-PGF2 in acute and exercise-induced asthma in children. Clin Exp Allergy 1998;28:181-6.
37. Hirawa N, Uehara Y, Yamakado M, Toya Y, Gomi T, Ikeda T, et al. Lipocalin-type prostaglandin d synthase in essential hypertension. Hypertension 2002;39:449-54.
38. Almishri W, Cossette C, Rokach J, Martin JG, Hamid Q, Powell WS. Effects of prostaglandin D2, 15-deoxy-Delta 12,14-prostaglandin J2, and selective DP1 and DP2 receptor agonists on pulmonary infiltration of eosinophils in Brown Norway rats. J Pharmacol Exp Ther 2005;313:64-9.
39. Ulven T, Kostenis E. Targeting the prostaglandin D2 receptors DP and CRTH2 for treatment of inflammation. Curr Top Med Chem 2006;6:1427-44.
40. Probst A, Spiegel HU. Cellular mechanisms of bone repair. J Invest Surg 1997;10:77-86.
41. Frost HM. The biology of fracture healing. An overview for clinicians. Part I. Clin Orthop Relat Res 1989;248:283-93.